Available online at www.sciencedirect.com

SciVerse ScienceDirect

Procedia Engineering 47 (2012) 1315 – 1318

Procedia Engineering

www.elsevier.com/locate/procedia

Proc. Eurosensors XXVI, September 9-12, 2012, Kraków, Poland

Preliminary studies on cell-free fetal DNA separation and extraction in glass lab-on-a-chip for capillary gel electrophoresis

Wojciech Kubicki, Rafał Walczak

Wrocław University of Technology, Janiszewskiego Str. 11/17, Wrocław 50-372, Poland

Abstract

Preliminary studies on separation and extraction of cell-free fetal DNA in glass lab-on-a-chip (LOC) prior to non-invasive prenatal diagnosis method, are presented. The chip, containing crossed injector, 25 mm long separator, Y-shaped extractor and glass reservoirs for fluid handling, was fabricated of borosilicate glass. DNA fragments in a size range 20-500 base pairs (bp), corresponding to fetal and maternal cell-free DNA, were separated and extracted in less than 2 minutes.

lab-on-a-chip; cell-free fetal DNA; electrophoresis; extraction

1. Introduction

Cell-free nucleic acids are present in circulating blood and are important markers of many clinical conditions, including strokes, tumor or cancer. In the case of pregnancies, cell-free fetal (cff) DNA isolated from maternal plasma has been used for non-invasive prenatal diagnosis and monitoring, e.g. detection of Down syndrome or hemolytic disease, as well as pregnancy confirmation and fetal sex determination. Cff DNA fragments usually cover size range < 300 base pairs (bp) and cell-free maternal DNA fragments are in majority > 500 bp [1,2]. As fetal genetic material represents only 3-6% of total maternal plasma cell-free DNA in circulatory, efficient extraction of cff DNA fragments (< 300 bp) from

* Wojciech Kubicki. Tel.: +48-71-355-98-66; fax: +48-71-328-35-04.
E-mail address: wojciech.kubicki@pwr.wroc.pl

doi:10.1016/j.proeng.2012.09.397

cell-free maternal DNA is required [3]. The other application includes extraction of specific fraction of separated genetic material for further analysis. Application of standard slab-gel instruments for this purpose is limited, due to high risk of DNA contamination during manual DNA extraction from the gel, as well as high sample consumption. Therefore, LOC-based methodology and instruments seem to be an alternative for traditional slab-gel.

The main goal of this work is development of LOC for extraction of DNA fragments (for example from maternal cell free genetic material or other "mixtures" of DNA) by the use of capillary gel electrophoresis (CGE). In the preliminary studies, separation and extraction of genomic DNA fragments of a size-range corresponding to the circulating cell-free fetal and maternal nucleic acids are separated and extracted in glass LOC with Y-shaped extractor.

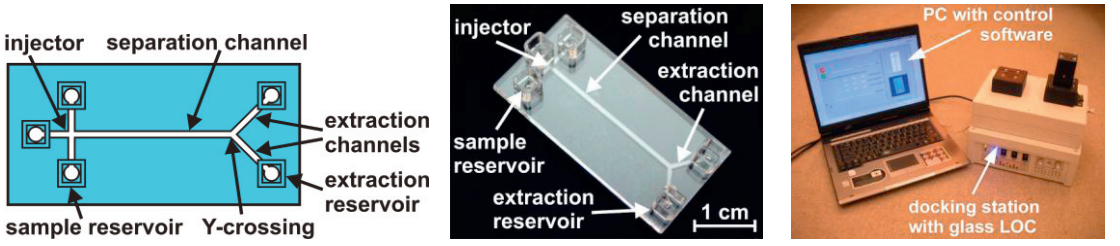
2. DNA sample

Experiments were performed for genomic and synthetic DNA fragments covering size range of both cell-free fetal and maternal genetic material. Shorter DNA fragments, corresponding to fetal DNA size, were prepared as 20-40 bp oligonucleotides and genomic 100, 200 and 300 bp fragments. Genomic 500 bp DNA fragments were used as counterpart of cell-free maternal DNA.

All DNA fragments were labeled with red-line fluorescence dye Cy5 (λ_{ex} = 640 nm, λ_{em} = 660-670 nm) utilizing PCR method.

3. LOC and instrument for CGE

The chip was fabricated of two borosilicate glass layers and five glass reservoirs, utilizing procedures of wet chemical etching, mechanical drilling and direct thermal bonding. LOC contains microfluidic cross-injector, 25 mm long separation channel, Y-crossing and two extraction channels (Fig. 1a). Glass reservoirs for fluid handling (approx. 36 µl) were place at the endings of microfluidic channels. Entirely glass construction of LOC provided excellent material compatibility with standard laboratory procedures, very good adjustment to sensitive fluorimetric detection method, as well as full reusability (Fig. 1b).



(a) (b) (c)

Fig. 1. All glass LOC and instrument for CGE: (a) topology of LOC; (b) ready-to-use glass LOC; (c) complete LOC instrument for CGE

DNA separation and extraction was performed in LOC instrument for CGE, described elsewhere [4] (Fig. 1c). The chip was placed in the docking station, providing electrohydrodynamic sample flow management in LOC, temperature regulation as well as genetic material detection by fluorimetric method with laser-induced fluorescence. The station was controlled by dedicated software for real-time data processing, display and storage. Smart processing algorithm with fluorescence signal feedback provided automatic injection, separation and extraction of DNA fragments in LOC.

4. Experiment and results

Microfluidic channels were filled with linear polymer sieving matrix (POL-4, A&A Biotechnology). Reservoirs were filled with standard running buffer (1x TBE, A&A Biotechnology). Wire electrodes were inserted into reservoirs. DNA sample was pipetted into reservoir No. 1 and afterwards all the operations were performed automatically (Fig. 2). DNA sample was injected into the separation channel utilizing pinched valve mechanism. Separation was performed applying high electric potential to extraction reservoirs. Extraction was obtained by accurate switching of electric potentials of reservoirs No. 4 and 5.

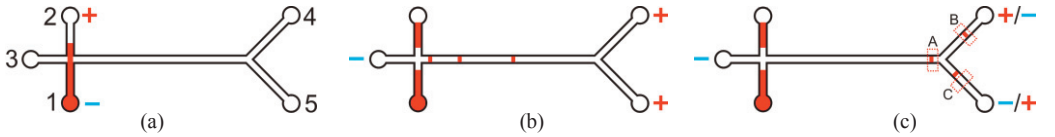


Fig. 2. Scheme of sample flow management in LOC: (a) transport; (b) injection and separation; (c) extraction of fractions into chosen extraction channels; A, B, C – detection areas

Laser beam (635 nm) was coupled through side-wall of the LOC to the centre of Y-crossing, perpendicularly to the separation channel. Y-crossing area was observed by CCD detector with low-pass optical filter (> 650 nm) for laser light blocking. Three independent detection areas (A, B, C) were defined in the recorded image. Detected fluorescence signals were used in feedback loop for voltage steering of reservoirs no. 4 and 5, as well as for processing into digital electropherograms. DNA fragments in a range 20-500 bp were successfully separated within 90 seconds (Fig. 3). Short DNA fragments (< 300 bp) were clearly separated from long (500 bp) fragments, indicating applicability of the chip for cff DNA extraction. The average separation efficiency for all the fractions was approx. 430 000 theoretical plates per meter. Presented solution provided easy extraction of DNA fragments selected by the user. It was found, that division and extraction of single DNA band was also possible (Fig. 4 b-d).

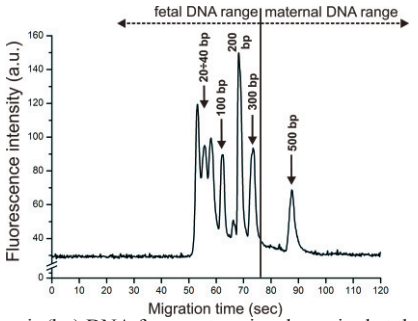


Fig. 3. Separation result of 20-500 base pair (bp) DNA fragments - signal acquired at the detection area A

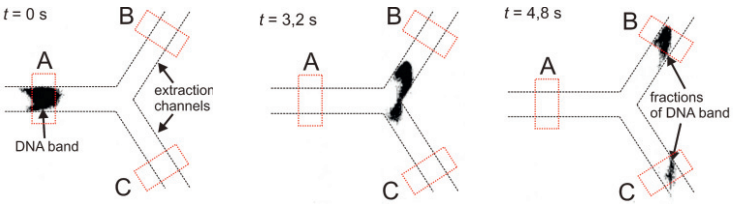
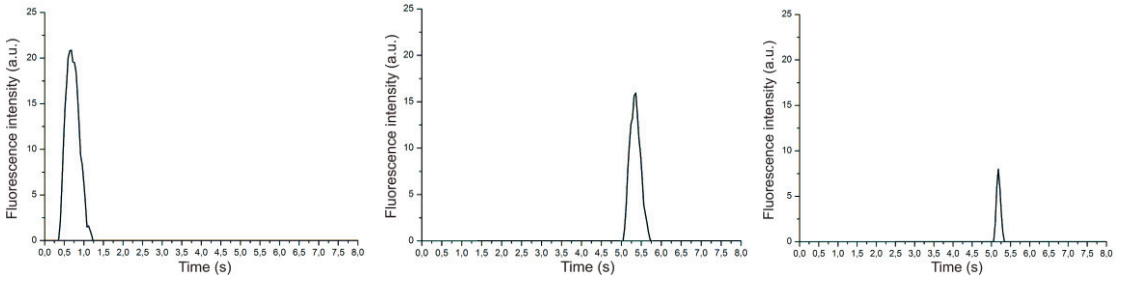


Fig. 4. Extraction of single DNA band (300 bp) at the Y-crossing as seen by the CCD-based fluorescence readout unit: (a) separated DNA band before Y-crossing; (b) band fractionation; (c) extraction of fractions; time count refers to appearance of DNA in area A

DNA fragment of 300 bp was successfully divided at the Y-crossing into two fractions utilizing accurate switching of electric voltage. Detection signal for DNA band and fractions was compared on the basis of fluorescence intensity plots (Fig. 5). The sum of fraction signals represented approx. 75% of the signal of the prime band. The difference is probably caused by nonuniform laser illumination of the extraction channels and therefore various excitation intensity of fluorochrome dye in extracted bands.



(a) (b) (c)

Fig. 5. Fluorescence intensity measured in detection area: a) A; b) B; c) C

5. Summary

Results of separation and extraction of DNA fragments in glass chip with Y-shaped extractor were presented. Genetic material of a size range 20-500 base pairs was successfully separated within 2 minutes. The average separation efficiency was estimated to 450 000 theoretical plates per meter. Sample flow in microfluidic channels of LOC was managed by software with feedback from three independent on-chip detection areas. This solution enabled efficient extraction of DNA fragments, as well as fractionation of a single DNA band. It opens the way for development of LOC-based apparatus for fast and reliable DNA extraction for further detailed analysis.

Acknowledgements

This work was supported by The National Centre of Research and Development (project No. O ROB 0031 01/ID/31/1).

References

[1] Maron J, Bianchi D. Prenatal diagnosis using cell-free nucleic acids in maternal body fluids: a decade of progress. *American Journal of Medical Gentics* 2007;**145C**:5-17.

[2] Bianchi D. Circulating fetal DNA: its origin and diagnostic potential – a review. *Placenta* 2004;**25**:93-101.

[3] Li Y, Holzgreve W, Hahn S. Size fractionation of cell-free DNA in maternal plasma and its application in noninvasive detection of fetal single gene point mutations. *Methods in Molecular Biology* 2008;**444**:239-251.

[4] Kubicki W, Walczak R, Dziuban J. Miniature instrument for lab-on-a-chip capillary gel electrophoresis of DNA utilizing temperature control technique. *Procedia Engineering* 2011;**25**:1237-1240.